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EYE TRACKING DYSFUNCTION IS A PUTATIVE PHENOTYPIC SUSCEPTIBILITY MARKER OF SCHIZOPHRENIA AND MAPS TO A LOCUS ON CHROMOSOME 6p IN FAMILIES WITH MULTIPLE OCCURRENCE OF THE DISEASE

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The difficulties in defining the borders of the schizophrenia spectrum is one major source of variance in linkage studies of schizophrenia. The employment of biological markers may prove advantageous. Due to empirical evidence, eye tracking dysfunction (ETD) has been discussed to be the most promising marker for genetic liability to schizophrenia. With respect to the recent progress in genomic scans, which have pointed to the short arm of chromosome 6, we carried out a scan of the 6p21-23 region with 16 microsatellite markers to test for linkage between chromosomal markers and ETD as well as schizophrenia. We tested 5 models of inheritance of ETD and found maximum two-point lod scores of 3.51 for D6S271 and 3.44 for D6S282. By including these markers in a multipoint analysis, a lod score of 4.02 was obtained. In the case of schizophrenia, 7 models were tested; however, with non-significant results. Our findings, together with another recent linkage report, point to the possibility of a second susceptibility locus for schizophrenia which may be located centromeric to the HLA region. Also, the evidence of ETD being a susceptibility marker for schizophrenia receives further support.

KEY WORDS: schizophrenia, linkage, eye tracking dysfunction, eye movements, biological markers

INTRODUCTION

Genetic-epidemiological studies of families, twins and adoptees have provided convincing evidence that genetic factors contribute substantially to the cause of schizophrenia [Kendler and Diehl, 1993]. Although molecular genetic technology has been rapidly progressing, research efforts have until recently not been

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successful in localizing chromosomal loci which might help to target potential susceptibility genes for schizophrenia. Two genome scans have failed to demonstrate significant results [Barr et al., 1994; Coon et al., 1994]. However in 1995, considerable evidence for linkage of schizophrenia to markers on chromosome 6p was gathered by 4 studies. Wang et al. [1995] performed a genome scan with linkage analysis in 186 families from Ireland, which had been recruited throughout the "Roscommon Study" [Kendler et al.,1993a], and obtained a lod score of 3.2 for the D6S260 locus. A multipoint lod score of 3.9 was achieved when the D6S260 and F13A1 loci were analyzed under the premise of heterogeneity. Straub et al. [1995] reported linkage results from 265 Irish pedigrees, which had been investigated in finer detail and of which the 186 pedigrees of Wang et al. are a subset. Straub et al. [1995] obtained a lod score of 3.51 for marker D6S296 and additional significant results with D6S274 and D6S285. These researchers also reported evidence for heterogeneity. Furthermore, Moises et al. [1995] proceeded with an two-stage genome-wide search, which was based on a multi-national cooperation. Among the 4 loci that were confirmed by the second stage study (D6S274, D6S291, D9S175, D20S40), 2 are situated on 6p. Schwab et al. [1995] screened 60 cM of 6p24-22 with 25 markers in 54 German and Israeli families. They performed a multipoint sib-pair linkage analysis and report a maximum lod score of 2.2 for D6S274 together with other positive lod scores which also indicate a susceptibility locus at 6p22-24. However, other attempts to detect linkage to markers in that region have failed [Antonarakis et al., 1995; Gurling et al., 1995; Mowry et al., 1995; Riley et al., 1995; Sasaki et al., 1995], although in one study some suggestion of linkage was observed around D6S296 [Antonarakis et al., 1995].

A number of methodical problems of linkage and association analyses have been elaborated [Elston, 1994; Risch, 1994, Suarez et al., 1994] that correspond to the suggested complexity of genetic transmission [Cardno and McGuffin, 1994; Kendler and Diehl, 1993; Prescott and Gottesman, 1993; Rutter, 1994]. The conflicting results of the recent studies of chromosome arm 6p might be due to several reasons, such as ethnic influence, size of pedigrees, and lack of statistical power in small family samples. It must be considered that diagnostic uncertainties can also be regarded as one major source of variability in linkage results [Hodge and Greenberg, 1992]. One problem is represented by the difficulties encountered in a valid definition of the limits of the schizophrenia phenotype. Establishing diagnoses is another obvious problem. Despite the substantial improvement of diagnostic reliability by modern diagnostic criteria, the rates of some of the schizophrenia spectrum disorders (schizotypal and paranoid personality disorders, non-schizophrenic non-affective psychosis) are widely variable across studies [Kendler and Diehl, 1993]. Recent linkage and association studies have consequently employed models of affectedness with differing breadth of inclusion criteria. It is unclear whether broad models of the schizophrenia spectrum which include all disease entities for which there is epidemiological evidence to be genetically linked to schizophrenia will be more successful than narrow models which only include schizophrenia and schizoaffective psychosis. While the study of Straub et al. [1995] argues for the first case, the work of Moises et al. [1995] points to the second, although these latter authors have only used a narrow model.

The existence of individuals who are diagnosed as unaffected on the basis of psychopathology, but are false-negatives, must be regarded as a major factor which contributes to variability of results in linkage studies. However, it is difficult to estimate its influence, since it is not known to what extent individuals in schizophrenic pedigrees are gene carriers although they fulfill only very few or even none of the diagnostic criteria to be included in one of the schizophrenia spectrum diagnoses. With respect to these difficulties, the employment of biological trait indicators can be of considerable advantage [Blackwood et al., 1991; McGuffin and Sturt, 1986; Baron, 1994].

The use of neurophysiological markers in the attempts to elucidate the genetics of schizophrenia has often been proposed [e.g. Baron, 1994; Holzman, 1992; 1994]. Eye tracking dysfunction (ETD) has been discussed as one of the most promising phenotypic markers for a genetic predisposition to this disease [recent reviews: Clementz and Sweeney, 1990; Holzman, 1992; Levy et al., 1993], due to a large body of empirical evidence. Inspired by the work of Holzman et al. [1973], a considerable number of studies on ETD has been performed during the last 2 decades. Not only schizophrenic index cases have been under investigation, but also first-degree relatives, twins and (to a smaller extent) groups of other psychiatric patients and from the general population. Although the methods of measurement and assessment of smooth pursuit eye movements have varied over the years, there is substantial accordance between the studies, especially when modern infrared technology and quantitative measures were used [Levy et al., 1993]. Sophisticated models of genetic transmission have been developed which have provided evidence that the genetic transmission of ETD in families with schizophrenia can be explained by a major gene model [Grove et al., 1992; Matthysse et al., 1986; Holzman et al., 1988].

Inspired by the recent reports on linkage of schizophrenia to markers on 6p and by our own collaboration with Moises et al. [1995] we conducted a search for linkage of ETD as well as different diagnostic categories of schizophrenia to markers of the p21-23 region of chromosome 6.

MATERIAL AND METHODS

Pedigrees and Diagnosis

The recruitment of families with multiple occurrence of schizophrenia (≥ 2 cases) was based on clinical chart records. In order to establish Axis I diagnoses (DSM-III-R), we used a German version of the Schedule for Affective Disorders and Schizophrenia/ Lifetime Version (SADS/L) [Endicott and Spitzer, 1978]. In addition, we employed the Structured Interview for Schizotypy (SIS) [Kendler et al., 1989], version 1.6, which was translated into German by two of us (V.A. and M.P.) for this study. The SIS provides detailed ratings of signs and symptoms which are common in the schizophrenia spectrum and therefore is suitable to establish the respective DSM-III-R personality disorders (schizotypal, paranoid, schizoid and avoidant personality disorder). It was one basic diagnostic instrument in the Roscommon Study [Kendler et al., 1993b] and therefore contributes to the diagnostic basis of the linkage findings of Straub et al. [1995] and Wang et al. [1995]. All psychiatric diagnoses of family members were established by personal interviews. The interviewers were blind to the diagnoses of relatives, but not of the index patients. They were also blind to genotypes and eye-movement

data. In addition to the personal interview with also included personal history, information was gathered from hospital records and family history. A consensus diagnosis could be established in all cases by 3 clinically experienced psychiatric interviewers after reviewing all available diagnostic data (V.A, M.P., A.N.).

Personal interviews and DNA was obtained from 73 individuals in 10 families. Of these, 7 members of one family disagreed with eye-movement recordings, and 4 members of another family had to be excluded, due to congenital nystagmus which made it impossible to assess smooth pursuit eye movements. The remaining 8 families with a total of 62 members were included in this study. None of these individuals had a diagnosis of substance abuse or was on medication with lithium containing compounds or benzodiazepines. Of the 17 schizophrenic family members, 15 were medicated with standard neuroleptics; the mean chlorpromazine dosage was 387.6 mg (SD: 423.3 mg) A clinical neurological examination which also included a detailed history, determinations of handedness, dominant eye, and fixation ability, did not reveal any sign of neurological disorder in any subject.

To test for linkage between schizophrenia spectrum disorders and genetic markers, we differentiated the psychiatric diagnoses into 3 categories which compare to those defined by Straub et al. [1995]. In category A (which refers to category "D1-D2" of Straub et al.] individuals with schizophrenia and schizoaffective psychoses were sampled. Differing from these researchers we did not dichotomize schizoaffective disorders into good and bad outcome subtypes. Further-more, no case of simple schizophrenia could be classified in our sample. Category B (which refers to category "D1-D5") adds to category A schizotypal personality disorder and nonaffective psychotic disorders. Category C (which refers to category "D1-D8") adds to category B mood incongruent and mood congruent affective disorders, but also paranoid, avoidant and schizoid personality disorder.

Assessment of ETD

Eye movements were recorded by a mobile infrared light (IR) eye-tracker (AMTech GmbH, Weinheim, Germany, and SKALAR, Delft, The Netherlands). With this device, we were able to examine individuals at home, who would otherwise have refused to participate. The IR high-resolution technique provides signal recordings with only few artifacts [Reulen et al., 1988]. The stimuli for eliciting smooth pursuit eye movements were displayed by an array of red light emitting diodes (LED). The target traverse consisted of 186 diodes subtending 30° (+/- 15°). The diodes (diameter = 5.7 mm, resp. 0.16°) were mantled in a white case as close as possible to guarantee a quasi-continuous target motion. For the same reason, two diodes might be energized during transition of the light signal, while the other diodes remained invisible. The experiments were carried out in darkened rooms. The results which were obtained by this mobile device are in accordance with results that were generated by a stationary device with a laser target (own unpublished data). Subjects were seated in a portable armchair 2 meters in front of the LED array. Their head was fixed by a helmet and they were instructed not to move their head during the measurement. Visual acuity was normal or corrected by individual glasses or contact lenses. IR recordings on the basis of the SKALAR system are not affected by eye glasses [Reulen et al., 1988].

In order to judge smooth pursuit

quality, we chose two triangular target movement paradigms with constant velocities of 15°/s and 30°/s. One trial lasted 20 sec plus 5 sec of calibration (steps of 10° from the central point to the left and the right side each lasting 1 sec) at the beginning and the end of each trial. After calibration of the original recordings, saccades were detected and counted. The definition of a saccade was based on 2 requirements: first, the difference between the actual velocity of the eye movement and the mean velocity before and after the fast eye movement had to be >30°/s, and secondly, the amplitude of the fast eye movement had to be >0.5°. Eye movement velocity was measured by using eye positions on the basis of a three-point central difference algorithm and a scanning frequency of 200 Hz (AMTech, Weinheim, Germany). Saccades in both directions of the target trajectory were detected. Also blinks were detected and cut out. In case of the rare event that a trial contained more than 6 blinks, it was completely omitted from the calculations. After having cut out saccades and artifacts, the mean eye velocity in each segment and the mean pursuit velocity gain (defined as ratio of the mean pursuit velocity to target velocity) were calculated in 5, respectively 10 cycles (5, resp. 10 segments with target motion to the right and 5, resp. 10 segments with target motion to the left side). A frame of 125 msec before and after the turning point was excluded from this procedure. To yield the average gain of the whole trial, the median of 9, respectively 18, segments was used. We used the median since it appeared to be the most robust measure. In a pre-study, we compared the median-based overall gain with time weighted average gain, but did not detect significant differences (unpublished data). A similar method had been employed in our pilot study [Moser et al., 1990], which also adheres to neurophysiological standards [Abel and Ziegler, 1988]. The quality of smooth pursuit was judged by the individual mean gain and the total number of saccades, after it had been compared to normal ranges for both parameters, established from a group of 47 controls. As controls, healthy students and medical staff members were chosen. In order to rule out an age effect on the assessment of smooth pursuit in the family members, the control sample was divided into three age groups: Group 1 (G1): 20-39 years, group 2 (G2): 40-59 years, group 3 (G3): ≥ 60 years.

We defined the thresholds for a smooth pursuit gain deficit as the arithmetic mean minus 2 SD for a target velocity of 15°/s and 30°/s, respectively. This definition of smooth pursuit deficit was also used in other studies on ETD [Levy et al., 1993]. Every performance with a mean gain below this threshold was rated as deviant eye tracking. Additionally, the limit for an abnormal number of saccades was defined as arithmetic mean plus 2 SD. Accordingly, every performance which led to a number of saccades above this limit was also rated as deviant. We included the saccadic threshold since there were individuals with clearly disturbed smooth pursuit which were still on the borderline of the normal range of mean gain. ETD was defined as present in an individual if the smooth pursuit performance yielded values either below the threshhold for mean gain or above the threshhold for mean number of saccades (Table I). We used the same procedure in a detailed analysis of ETD in families with multiple occurrence of schizophrenia [Arolt et al., 1996] which was in accordance with another study in which qualitative global eye movement measures and personal ratings were used [Myles-Worsley et al., 1992]. Thus, 4 different ratings (2 measures each from 2 paradigms) were employed, in

TABLE I: Thresholds (in italics) for the Assessment of Eye Tracking Dysfunction, Based on Quantitative Measures. The Target Velocity v = 15°/s and v = 30°/s.*

v =15°/s age	mg	*mg - 2SD*	sacc	*sacc +2 SD*
20-39	0.95	*0.76*	28.0	*43.3*
30-59	0.95	*0.76*	36.6	*58.5*
> 60	0.88	*0.75*	44.3	*72.1*
v =30°/s age	**mg**	***mg 2SD***	**sacc**	***sacc + 2 SD***
20-39	0.82	*0.63*	45.3	*72.0*
30-59	0.75	*0.62*	55.8	*87.4*
> 60	0.72	*0.49*	67.6	*106.6*

* mg: mean pursuit gain; sacc: number of saccades/ 20s

order to provide a reasonable broad spectrum of smooth pursuit dysfunction assessment. Accordingly, we checked for the possibilty of false positive, resp. false-negative decisions on the ETD status, which can be expected if a measure is close to the 2 SD threshold. If one parameter was situated in the range of 2 SD - 2.5 SD, we suspected a possible false positive result; a false negative result was suspected if a range of 1.5 SD - 2.0 SD was met. The following results were obtained: 1. (possible false positive case). Of the 63 probands, there were 10 in which one of the measures was situated in the 2 SD - 2.5 SD range. Of these, 7 were characterized by at least one parameter > 2.5 SD and were rated as having ETD. In the remaining 3 cases, 2 parameters met the range. The possibility of false positive error was neglected in these cases and ETD was assumed. 2. (possible false negative case). There were 20 cases in which one parameter fell into the 1.5 SD - 2.0 SD range. Of these, 14 performed with at least one measure > 2.5 and hence were rated as ETD positive. In the remaining 6 cases all 3 other parameters were below 1.5; these individuals were rated as ETD negative. Accordingly, ETD was assumed if either one parameter was rated > 2.5 SD, or if 2 parameters were > 2.0.

Molecular Genetic Methods

DNA was isolated from whole blood samples using standard protocols. Marker alleles were amplified by PCR basically following the method of Weber and May [1989]. PCR products were size-separated on 8% polyacrylamide gels and visualized by silver staining [Budowle et al., 1991]. Genotypes were read independently by two researchers who were not informed about psychiatric diagnoses or eye movement status of the subjects. Sixteen microsatellite markers were chosen from the Généthon map [Gyapay et al. 1994]: D6S105, D6S259, D6S260, D6S271, D6S274, D6S276, D6S282, D6S285, D6S288, D6S291, D6S299, D6S426, D6S451, D6S452, D6S459, and D6S465. Details about primer sequences and genetic distances between marker loci were from the same

TABLE II: Inheritance Models for Eye Tracking Dysfunction

	Allele frequency A	Penetrance rates AA	Aa	aa	Phenocopy rate (%)	Prevalence rate* (%)
I	.010	.94	.80	.030	64,8	4,5
II	.010	.90	.70	.045	76,0	5,8
III	.030	.90	.70	.018	29,0	5,8
IV	.010	.98	.90	.042	70,0	5,9
V	.030	.98	.90	.006	10,0	5,9

* estimated prevalence of ETD in the general population

source and from the CEPH genotype database (www.cephb.fr).

Assessment of Inheritance of ETD and Schizophrenia

Our analyses were based on autosomal close to dominant major gene models for ETD which, on the basis of empirical findings, seemed to fit best the genetic transmission of ETD (Table II). By using complex segregation analysis, Grove et al. [1992] demonstrated that a mixed model yielded the best explanation for the segregation patterns in families with ETD. The pattern of inheritance of the major gene did not completely fit either a recessive or a dominant model. In adaptation of these findings we developed our first inheritance model (I). Inspection of our pedigrees gave the impression of possible dominant inheritance. Therefore, we established an intermediate, but close to dominant model of inheritance, by gauging penetrances and allele frequencies to fit a population prevalence of 5%. This prevalence rate was obtained from the largest study of non-schizophrenic individuals and their relatives [Iacono et al., 1992]. We developed 4 other models (II-V), for which we estimated a prevalence rate of about 6% for ETD in the general

TABLE III: Inheritance Models for Schizophrenia.

	Allele frequency (A)	Penetrance rates AA	Aa	aa
A0, dominant	.01	.235	.20	.00625
A1, dominant ("dom D1-D2")	.0049	.55	.55	.0006
A2, intermediate ("pen D1-D2")	.0098	.55	.275	.0006
B1, dominant ("dom D1-D5")	.0161	.75	.75	.0062
B2, intermediate ("pen D1-D5")	.0320	.75	.375	.0064
C1, dominant ("dom D1-D8")	.0178	.85	.85	.0104
C2, intermediate ("pen D1-D8")	.0353	.85	.425	.0108

n.b.: the following categories from the schizophrenia spectrum were used in the models: A: narrow model = schizophrenia and schizoaffective psychosis, B: extended model = A + schizotypal personality disorders + non-affective psychoses, C: broad model = B + affective disorders, paranoid, schizoid and avoidant personality disorders. Quotation marks refer to inheritance models from the report of Straub et al. [1995].

population, due to our own observation in healthy controls (6.3%, unpublished data). This assumption is also in accordance with the literature on ETD in healthy individuals, in which rates between 4 and 8% have been demonstrated [Levy et al., 1993]. We have varied our initial model with regard to different rates of phenocopies and gene frequencies (Table II) in a stepwise procedure, which we chose since neither of these parameters can be precisely determined. In estimating phenocopy rates, we had to account for the fact that ETD is not only observed in schizophrenia, but also in a variety of neurological disorders. It may also be the case that ETD might be coded on other genomic regions than those which have been screened in this study. However, it is to date impossible to refer to precise estimates. Based on these considerations we decided to employ two different penetrance rates (high: models II and IV; low: models III and IV). With regard to allele frequency, it was our intention to vary the degree of dominance. In models II and III, we employed lower penetrance rates than in our initial model, but varied gene frequency (and rate of phenocopies, respectively). In models IV and V the same procedure was used, but with higher penetrance rates (Table II).

Furthermore, we tested for linkage to schizophrenia by employing different diagnostic categories from the schizophrenia spectrum (see "Pedigrees and Diagnoses" in this section). While one of the inheritance models was developed by ourselves (narrow model A0), the other 6 were taken from the article of Straub et al. [1995], in order to obtain results which are easily compared across studies (Table III).

The linkage analyses were calculated by using the FASTLINK program [Cottingham et al., 1993; Schaffer et al., 1994] which is based on the LINKAGE package [Lathrop et al., 1984]. In addition to regular linkage analysis, we also applied the admixture test for locus heterogeneity as implemented in HOMOG [Ott, 1991].

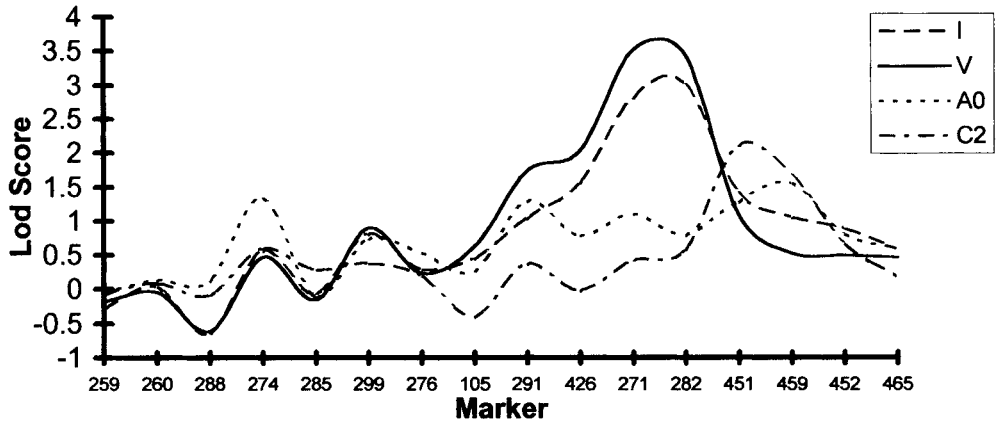

Fig.1: Two-point lod scores for ETD (inheritance models I and V) and schizophrenia (inheritance models A0 and C2).Genetic models are specified in tables II and III. Maximum lod scores were chosen at recombination fractions (θ) 0.00 to 0.20. The sequence of the markers was defined according to the Généthon map [Gyapay et al., 1994] and the CEPH genotype database (www.cephb.fr).

RESULTS

The following psychiatric diagnoses were obtained from the 63 family members of 8 families: schizophrenia (n = 17) schizoaffective psychosis (n = 4), non-affective, non-schizophrenic psychosis (n = 1), major depressive disorder (n = 4), bipolar affective disorder (n = 1), other Axis I diagnoses (n = 1), schizotypal personality disorder (n = 4), paranoid personality disorder (n = 1), schizoid personality disorder (n = 4), dependent personality disorder (n = 1), other personality disorders (n = 4), no psychiatric diagnoses (n = 21). Of these 62 family members, 39 had ETD (61,9%). There was a high degree of concordance between cases of schizophrenia and ETD (16/17), schizoaffective psychosis and ETD (3/4), and non-affective, non-schizophrenic psychosis (1/1).

In order to test for linkage, we calculated two-point linkage analyses between ETD and 16 microsatellite markers on chromosome 6p21-23 (Table IV). The best results were obtained for D6S271 (maximum lod score of 3.51 at $\theta = 0.0$) and D6S282 (maximum lod score of 3.44 at $\theta = 0.05$) in model V (Fig. 1). The other models yielded smaller, however suggestive or significant lod scores (Table IV). In a second step we calculated multipoint analyses by including the most promising markers D6S271 and D6S282, which map closely on chromosome 6p. The multipoint analysis yielded a maximum lod score of 4.04 on the basis of model V (Table V). A qualitative inspection of the pedigrees demonstrated cosegregation without recombination of ETD and respective alleles in 4 families (2, 5, 7, 13) for markers D6S271 and D6S282 (Table IV). While in these families the highest lod scores were

TABLE IV: Two-point lod Scores for ETD and Schizophrenia vs. Chromosome 6p Markers.

	Genetic models											
	ETD					Schizophrenia categories						
							D1-D2		D1-D5		D1-D8	
	I	II	III	IV	V	A0	A1	A2	B1	B2	C1	C2
Markers												
D6S259	-0.30	-0.02	-0.21	-0.30	-0.18	-0.08	-0.10	-0.07	-0.34	-0.16	-0.23	-0.09
D6S260	0.02	0.14	0.07	-0.04	-0.05	0.12	0.03	0.05	0.22	0.30	-0.55	0.07
D6S288	-0.66	-0.10	-0.51	-0.65	-0.61	0.10	0.26	0.09	0.39	0.30	-0.05	-0.11
D6S274	0.55	0.65	0.62	0.43	0.47	1.33	0.84	1.15	0.75	1.41	-0.05	0.58
D6S285	-0.08	-0.01	-0.09	-0.07	-0.16	-0.12	-0.45	-0.28	-0.57	-0.35	o.11	0.27
D6S299	0.80	0.73	0.79	0.81	0.90	0.71	0.44	0.55	0.36	0.42	0.20	0.37
D6S276	0.28	0.32	0.38	0.16	0.23	0.53	0.50	0.58	-0.30	-0.03	-0.28	0.19
D6S105	0.43	0.36	0.48	0.47	0.63	0.24	0.38	0.23	0.13	0.20	-0.82	-0.41
D6S291	1.02	1.00	1.39	1.12	1.73	1.28	1.34	1.33	0.90	0.66	-0.02	0.36
D6S426	1.53	1.40	1.65	1.70	2.03	0.77	1.06	0.96	-0.01	-0.01	-0.53	-0.03
D6S271	2.79	2.48	2.93	2.91	3.51	1.08	1.32	1.22	0.81	0.74	0.22	0.40
D6S282	3.02	2.77	2.97	3.19	3.44	0.78	0.79	0.79	0.99	0.79	0.16	0.55
D6S451	1.42	1.87	1.88	0.93	1.08	1.27	1.15	1.44	0.29	0.88	0.68	2.08
D6S459	1.04	1.10	0.98	0.96	0.52	1.54	1.38	1.75	1.30	1.25	1.87	1.68
D6S452	0.88	1.05	0.89	0.93	0.49	0.80	0.70	0.84	0.40	0.61	-0.01	0.66
D6S465	0.57	0.70	0.66	0.54	0.46	0.58	0.73	0.67	0.30	0.41	-0.38	0.17

n.b.: genetic models are specified in Tables II and III. Maximum lod scores were chosen at recombination fractions (θ) 0.00 to 0.20. The sequence of the markers was defined according to the Généthon map [Gyapay et al., 1994] and the CEPH genotype database (www.cephb.fr)

TABLE V: Maximum Two- and Three-point lod Scores at Recombination Fractions for ETD and Markers D6S271 and D6S282 on the Basis of Different Models of Inheritance (Table III).

Model	D6S271 pairwise lod (θ)	D6S282 pairwise lod (θ)	Threepoint maximum lod
I	2.79 (.03)	3.02 (.05)	3.39
II	2.48 (.01)	2.77 (.05)	3.07
III	2.93 (.00)	2.97 (.03)	3.34
IV	2.91 (.03)	3.19 (.05)	3.48
V	3.51 (.00)	3.44 (.05)	4.02

obtained, the scores from the other families (3, 9, 10, 11) were negative or close to zero. The pedigrees with higher lod scores were more informative, since the number of close relatives was higher than in the other families. No differences with respect to concordance of ETD and schizophrenia could be observed.

We also tested for linkage between models of different breadth of the schizophrenia spectrum, however, without significant results (Table IV). By our own narrow inheritance model (A0), lod scores of 1.16 at $\theta = 0.0$ for D6S271 and 0.76 at $\theta = 0.13$ for D6S282 were obtained. Again, as in the case of ETD, no recombinations and lod scores of 1.78 and 1.77 at $\theta = 0.0$ were observed for markers D6S271 and D6S282 in families 2,5,7 and 13. We also tested other categories covering the schizophrenia spectrum under the assumption of different models of inheritance (A1, A2, B1, B2, C1, C2). There was no verification of linkage whatsoever, and none of the lod scores obtained was as large as the lod scores that were obtained for ETD with markers D6S271 and D6S282 (Table IV; Fig.1). A formal test for heterogeneity by using HOMOG [Ott, 1991] gave no significant evidence in favor of the involvement of another locus (likelihood ratio of 1.02).

DISCUSSION

Due to a large body of empirical evidence ETD is conceived as a putative phenotypic marker for genetic susceptibility to schizophrenia. This study is the first attempt to test for linkage between ETD and DNA markers in families with a suspected high genetic predisposition for schizophrenia. In contrast to all other linkage and association studies published to date, we did not primarily employ different definitions of the schizophrenia spectrum, but focused on a biological marker. A scan was carried out which was strictly limited to the chromosomal region 6p21-23. By carefully extending our initially used model of inheritance of ETD [Arolt et al., 1996], we have obtained suggestive evidence that ETD maps to markers at 6p21. With respect to the proposals of Lander and Kruglyak [1995] and Thomsen [1995], we think that our findings can be interpreted as significant linkage for the two-point and the multipoint analyses on the basis of model V (high penetrance, low phenocopy rate).

Table VI: Familywise lod Scores From a Two-point Analysis (model V) of ETD vs. 2 Markers on Chromosome 6p21 in 8 Families.

Family No.	Markers	
	D6S271 θ = .00	D6S282 θ = .05
Total	3.51	3.44
2	*1.08*	*0.98*
3	0.00	-0.26
5	*1.07*	*0.92*
7	*1.01*	*0.90*
9	-0.23	-0.32
10	*0.32*	0.11
11	-0.83	0.13
13	*1.09*	*0.99*

n.b. italics = numbers of families (and corresponding lod scores) in which cosegregation of ETD and markers without recombination was observed.

The results of this study indicate that a locus in the 6p21 region may contribute to the genetic predisposition to schizophrenia. We found evidence for linkage in a chromosomal region that is situated close to the regions that have been highlighted by the recent studies of linkage to schizophrenia. It must be acknowledged that most of these findings point to a region of about 20 centimorgans which is located distal to the HLA region and in the direction of the telomere [Antonarakis et al., 1995; Moises et al., 1995; Schwab et al., 1995; Straub et al., 1995; Wang et al., 1995], while our results concern markers which are situated proximal of the HLA region. However, it is noteworthy that one of the four confirmed loci in the study of Moises et al. [1995] was marked by D6S291, which is close to the region implicated by our findings (Fig. 1). Hence it can be hypothesized whether there are two susceptibility loci on chromosome 6p, flanking the HLA region.

When we tested for linkage between models of different breadth of the schizophrenia spectrum and our set of markers, we did not observe any suggestive results, although we have used similarly defined categories and the same genetic models as Straub et al. [1995] and Moises et al. [1995], respectively. On the other hand, our linkage analysis did not yield strongly negative lod scores. Therefore, we have neither been able to confirm nor to exclude recent findings of linkage to schizophrenia on the short arm of chromosome 6. Since a conceivable reason for these results is the lack of statistical power, due to small sample size, we think that it would be unwise to draw firm conclusions on linkage from these data.

What is the reason why we detected significant lod scores for ETD, although the sample size was small, and why are these lod scores substantially higher than those obtained for schizophrenia? As in other studies on ETD in relatives of schizophrenic probands [Levy et al., 1993], we demonstrated in another study that in families with multiple occurrences of schizophrenia affected individuals uniformly manifest ETD, as well as a number of relatives without significant psychopathlogy [Arolt et al., 1996]. In the families selected for linkage studies, the proportion of individuals which are falsely classified as genetically unaffected on the basis of psychopathology would probably be smaller if instead ETD was employed as a trait marker. This effect can be suspected to be the more significant, the larger the number of psychopathologically inconspicuous susceptibility gene carriers is assumed in a family. It might be the case that ETD is a better phenotypic expression of a genetic predis-

position for schizophrenia than psychopathological symptoms, and accordingly, the reduction of the proportion of false negatives might have strongly contributed to the significance of our findings. We also found a high degree of concordance between schizophrenia (and related psychotic disorders) and ETD in our families. Therefore, we did not additionally test a linkage model in which subjects with ETD and subjects with schizophrenia are combined (if considered as two phenotypic expressions of a genotype).

We can not completely rule out the possibility of a false positive linkage result since, due to the lack of respective empirical findings in ETD research, a misclassification of allele frequencies cannot be excluded. The chance of such misclassification can be reduced when different models are calculated. On the other hand, it was one of our intentions to avoid the perils of multiple testing. The validity of our results is supported by our decision to use only one definition of ETD and only 5 models of inheritance, which are in accordance with eye movement research. However, we had to make allowances for the fact that only little information about the mode of inheritance of ETD is provided in the literature. It can be gathered from what has been published that we possibly face different genetic assumptions in the case of ETD than in the case of schizophrenia. The transmission of ETD seems to be less complex. There is some evidence that ETD might be inherited in a mendelian fashion by a single gene [Matthysse et al., 1986; Holzman et al., 1988] or a major gene model [Grove et al., 1992]. On the other hand, while we were able to observe cosegregation with the respective genotype markers in 4 of 8 families, we did not detect this phenomenon in the other 4 families. This finding points to the possibility that the transmission of ETD in families with multiple occurrence of schizophrenia might be heterogeneous. However, when we tested for heterogeneity, this yielded a non-significant result. It should also be considered in this respect that those families in whom only small or negative lod scores where obtained, were the genetically least informative, due to relatively distant relationships.

Another problem for genetic modeling is represented by the fact that the prevalence rate of ETD in the general population is not precisely known. We decided to choose a rate of 6%, which is derived from our own observation and which fits into the variation which can be found in the literature. We have also been confronted with the problem that the rate of phenocopies is uncertain in the case of ETD. It must be considered in this respect that a number of neurological diseases, but also substances (e.g. diazepam and its derivates, lithium, alcohol) cause saccadic disruptions of smooth pursuit which may be indistinguishable from what we assume is genetically based. It may also be the case that other loci are involved in a similar type of ETD, which would also contribute to the rate of phenocopies. With regard to these problems which to date cannot be solved on the basis of empirical data, we decided to use two variations of the phenocopy rate (low: ≈10-30%; high: ≈65-75%). The best result was yielded by the model in which the lowest phenocopy rate and the highest penetrances were incorporated (model V). This finding, though not statistically significant, may indicate that the locus found to be involved is, at least in our families, responsible for a larger part of the variation in the trait ETD than we had initially assumed. It is in fact our impression that a dominant mode of inheritance could be the most suitable model for our families.

Although other groups used similar methods of assessment of ETD

in many respects, it must be noted that a variety of measures has been employed in eye movement research in psychiatric patients. Qualitative measures (rating scales) or global quantitative measures (ln S/N and root-mean-square) have played a major role, but during the last decade an increasing number of researchers have relied on quantitative measures, such as mean gain and number of saccades [Levy et al., 1993]. We have also employed these two measures but additionally dichotomized into normal and abnormal eye tracking. We decided for this procedure since it was not only based on measures which are accepted as standards in neurophysiology, but also since we were able to demonstrate that the distribution of ETD (according to our definition) in families with multiple occurrence of schizophrenia covers nearly all schizophrenia spectrum cases, and also psychopathologically inconspicuous relatives [Arolt et al., 1996]. The validity of our procedure to assess ETD is supported by the similarities that can be observed in large Palauan pedigrees, although in this study global measures of ETD were used [Myles-Worsley et al., 1992]. However, there have also been findings, that ETD (assessed by mean gain and root-mean-square) might not be present in all families with schizophrenia [Clementz et al., 1992].

Since our results are based on a relatively small sample of 8 German families, it may be doubted that they are representative of families with multiple occurrence of schizophrenia. To confirm these data it is necessary to replicate our findings in a larger number of families. On the other hand, such confirmation would also considerably support the understanding of ETD to represent a phenotypic marker of genetic susceptibility to schizophrenia.

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